

Applicant : Jason Koontz et
Serial No. : 09/874,162
Filed : June 4, 2001
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Attorney : [REDACTED] Docket No.: 05311-024001

REMARKS

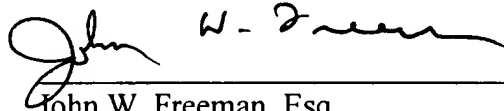
Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 1/9/02



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"Version With Markings to Show Changes Made"

In the specification:

Paragraph beginning at page 52, line 17, has been amended as follows:

The genomic sequence available for BAC b307A16 was filtered using the RepeatMasker2 program and the resulting repeat-free sequence was used to search the GenBank and EST databases for DNA having intron-exon structure. A complex EST, KIAA0160, with a total 4.44 kb of sequence was found to map in genomic DNA less than 20 kb away from the chromosome 17 breakpoint; however, the first 647 bp of this EST was not present from any portion of b307A16 or the overlapping BAC b542B22. This suggested that an interstitial deletion had occurred in the genomic DNA contained in b307A16 and that the predicted gene extends beyond the chromosome 17 breakpoint. If this gene were disrupted by the chromosome 17 breakpoint, the orientation of the gene would be consistent with a fusion of this gene with the gene disrupted by the breakpoint in chromosome 7. To test this possibility, RNA derived from tumor BWH-42 and RNA from control fibroblasts was analyzed by RT-PCR using primers complementary to sense sequence at the 5' end of both the 7 and 17 genes and anti-sense sequence located at the 3' end of both genes, with the primers paired in all four possible ways. RT-PCR analysis was performed as follows. RNA derived from either the primary tissue culture cells or the frozen surgical specimens was transcribed into cDNA with Superscript II (Life Technologies) according to the supplier's instructions, using either 7 AntisenseOuter or 17 AntisenseOuter as a primer (see below). The resulting cDNA was subjected to two rounds of PCR (98° C for 5 minutes; 10 cycles of 98° C for 15 seconds, 65° C minus 0.5° C per cycle for 30 seconds, 74° C for 1 minute; 15 cycles of 98° C for 15 seconds, 60° C for 30 seconds, 74° C for 1 minute) using first the "Outer" and then the "Inner" set of primers. The *JAZF1* primers were 7SenseOuter 5'-CCACAGCAGTGGAAGCCTTA-3' (SEQ ID NO:10), 7AntisenseOuter 5'-GCTTCTCTTCCCCTCCATTCAT-3' (SEQ ID NO:11), 7SenseInner 5'-ATCACCCCCTCCTCTTCATT-3' (SEQ ID NO:12), and 7 AntisenseInner 5'-GGACTCATCGCTGTCCGACT-3' (SEQ ID NO:13). The *JJAZ1* primers were 17SenseOuter 5'-GTTACTCGGCCTCCTCCTCCTC-3' (SEQ ID NO:14), 17 AntisenseOuter 5'-GGTTCAAATTCATTACTGGAAACTGC-3' (SEQ ID NO:15), 17 SenseInner 5'-

GAGCTTTTCCTCCAGGCCTTTG-3' (SEQ ID NO:16) and 17 AntisenseInner 5'-CCGGGTTTTGTTTGATTGAGG-3' (SEQ ID NO:17). Specific primers for glyceraldehydes 3-phosphate dehydrogenase (GAPF 5'-CACATCGCTCAGACACCATG-3' (SEQ ID NO:18) and GAPR 5'-GCCATGGAATTTGCCATGGG-3' (SEQ ID NO:19)) were used to assess the quality of the input RNA.

Paragraph beginning at page 53, line 17, has been amended as follows:

In these experiments, the primer pairs for the intact chromosome 17 gene amplified products from the RNA of both the normal tissue and the tumor, but the recombinant 5' chromosome 7 - 3' chromosome 17 pair amplified a product of 440 bp only from the tumor RNA and not from the normal RNA. The reciprocal recombinant primer pair failed to amplify a product from either of the RNA samples. 5' chromosome 7 - 3' chromosome 17 primer pair was then used in an RT-PCR assay of RNA derived from all four tumors used previously in Northern blot analyses and an identical product was obtained in all four samples. RT-PCR analysis of *JAZF1-JJAZ* expression in formalin-fixed, paraffin-embedded material was carried out using protocols similar to those for amplification of the RNA sequences for the individual genes, except for preparation of the RNA template. Total RNA was extracted from ten serial 10 µm tissue sections with 3 washes in 10 ml of xylene. An adjacent 10 µm tissue section was placed on a microscope slide and stained with hematoxylin and eosin for histologic examination. For extraction of RNA, tissue was rehydrated in two washes with 100% EtOH and was digested for 16h at 60° C in 2 ml 1x digestion buffer (20 mM Tris-HCl pH 8.0, 20 mM Na₂EDTA, 2% SDS, 2.5 mg/mL proteinase K). The lysate was diluted with 8 ml Trizol and the RNA isolated according to the supplier's instructions. First-strand synthesis and nested PCR were performed as described above with the following primers: FusionOutF 5'-CACGCCACAGCAGTGGAAGC-3' (SEQ ID NO:20), FusionOutR 5'-TTTGTCTCTGGAGTTTCGATGAGACA-3' (SEQ ID NO:21), FusionInnerF 5'-CCCACCCATCACCCCTCCT-3' (SEQ ID NO:22), and FusionInnerR 5'-GGTGCTATGAGATTCCGAGTTCGAAGA-3' (SEQ ID NO:23). These results confirmed that the chromosome 17 gene was disrupted by the translocation and that a fusion transcript

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containing the 5' end of the chromosome 7 gene and the 3' end of the chromosome 17 gene had been created.